

09/13/93 386

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**Term:**

L7 and (immobiliz\$5 or attach\$4)

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result set

DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ

<u>L8</u>	L7 and (immobiliz\$5 or attach\$4)	10	<u>L8</u>
<u>L7</u>	(end\$1 or termin\$3) near5 cleav\$3 near5 (phosphorothioate\$1 or phosphoramidate) near5 (primer\$1 or probe\$1 or oligonucleotide\$1)	12	<u>L7</u>
<u>L6</u>	(end\$1 or termin\$3) near5 cleav\$3 near5 (phosphorothioate\$1 or phosphoraidate\$1) near5 primer\$1	1	<u>L6</u>
<u>L5</u>	L4 and ((modif\$3 near5 base) or (modif\$3 near5 sugar) or (modif\$3 near5 phosphate backbone))	32	<u>L5</u>
<u>L4</u>	L3 and polymerase chain reaction\$1	48	<u>L4</u>
<u>L3</u>	L2 and (end\$1 or termi\$4)	81	<u>L3</u>
<u>L2</u>	L1 and (immobiliz\$5 or attach\$4)	82	<u>L2</u>
<u>L1</u>	(phosphorothioate or phosphoramidate)near5(primer\$1 or oligonucleotide\$1 or probe\$1) near5 cleav\$4	95	<u>L1</u>

09/139.386

=> s (phosphorothioate# or phosphoramidate#) (10a) (end# or termin###) (10a) primer#  
 L1 17 (PHOSPHOROTHIOATE# OR PHOSPHORAMIDATE#) (10A) (END# OR TERMIN###) (10A) PRIMER#

=> s l1 and clea#### and ligand#  
 L2 0 L1 AND CLEA#### AND LIGAND#

=> s l1 and cleav####  
 L3 1 L1 AND CLEAV####

=> s l3 and (immobiliz##### or attach### or ligand#)  
 L4 0 L3 AND (IMMOBILIZ##### OR ATTACH### OR LIGAND#)

=> d l3 bib ab kwic

L3 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2003 ACS on STN  
 AN 1996:494192 CAPLUS  
 DN 125:134793  
 TI Synthesis of stereospecific oligonucleotide phosphorothioates and antisense oligonucleotides  
 IN Tang, Jinyan; Roskey, Allysen M.; Agrawal, Sudhir  
 PA Hybridon, Inc., USA  
 SO PCT Int. Appl., 37 pp.  
 CODEN: PIXXD2  
 DT Patent  
 LA English  
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9619572	A1	19960627	WO 1995-US16086	19951212
	W: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, FI, GB, GE, HU, IS, JP, KE, KG, KP, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	CA 2208528	AA	19960627	CA 1995-2208528	19951212
	AU 9645146	A1	19960710	AU 1996-45146	19951212
	EP 807171	A1	19971119	EP 1995-943748	19951212
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE				
	CN 1175281	A	19980304	CN 1995-197690	19951212
PRAI	US 1994-362631		19941222		
	WO 1995-US16086		19951212		

AB Disclosed is a method for synthesizing of stereospecific (Rp) phosphorothioate oligonucleotides. In this method, a primer comprising a plurality of deoxyribonucleotides and a ribonucleotide at the 5' terminal or 5' penultimate position, is annealed to a template. The structure is contacted with a mixt. of deoxynucleoside .alpha.-triphosphate Sp diastereomers and a DNA polymerase to form a PS-Rp oligodeoxynucleotide extension which is liberated as a single-stranded PS-Rp oligonucleotide by **cleavage** after the ribonucleotide in the primer. Also disclosed are PS-RP oligonucleotides and oligonucleotides prepd. according to this method.

AB Disclosed is a method for synthesizing of stereospecific (Rp) phosphorothioate oligonucleotides. In this method, a primer comprising a plurality of deoxyribonucleotides and a ribonucleotide at the 5' terminal or 5' penultimate position, is annealed to a template. The structure is contacted with a mixt. of deoxynucleoside .alpha.-triphosphate Sp diastereomers and a DNA polymerase to form a PS-Rp oligodeoxynucleotide extension which is liberated as a single-stranded PS-Rp oligonucleotide by **cleavage** after the ribonucleotide in the primer. Also disclosed are PS-RP oligonucleotides and oligonucleotides prepd. according to this method.

IT 179312-03-9D, phosphorus-32-end labeled 179312-05-1

RL: NUU (Other use, unclassified); USES (Uses)

(**primer**; synthesis of stereospecific oligonucleotide  
**phosphorothioates** and antisense oligonucleotides)

IT 1310-58-3, Potassium hydroxide, uses 1310-65-2, Lithium hydroxide  
1310-73-2, Sodium hydroxide, uses 1336-21-6, Ammonium hydroxide  
9026-12-4, Rnase t1 37205-57-5, Rnase u2 37278-25-4, Rnase t2

RL: NUU (Other use, unclassified); USES (Uses)

(template **cleavage** from primer; synthesis of stereospecific  
oligonucleotide phosphorothioates and antisense oligonucleotides)

=> s (phosphorothioate# or phosphoramidate#)(10a)primer#

L5 115 (PHOSPHOROTHIOATE# OR PHOSPHORAMIDATE#)(10A) PRIMER#

=> s 15 and ligand#

L6 2 L5 AND LIGAND#

=> s 16 and cleav####

L7 0 L6 AND CLEAV####

=> s 15 and cleav####

L8 20 L5 AND CLEAV####

=> s 18 and ligand#

L9 0 L8 AND LIGAND#

=> s 18 and (immobiliz##### or attach#####)

L10 10 L8 AND (IMMOBILIZ##### OR ATTACH#####)

=> dup rem l10

PROCESSING COMPLETED FOR L10

L11 10 DUP REM L10 (0 DUPLICATES REMOVED)

=> d l11 bib ab kwic

L11 ANSWER 1 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2003:196911 CAPLUS

DN 138:232931

TI Multiplex anchored strand displacement amplification on an electronically  
addressable microchip

IN Nerenberg, Michael I.; Edman, Carl F.; Westin, Lorelei P.; Feng, Lana L.;  
Landis, Geoffrey C.; Sosnowski, Ronald G.

PA Nanogen/Becton Dickinson Partnership, USA

SO U.S., 77 pp.

CODEN: USXXAM

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 6531302	B1	20030311	US 1999-290000	19990412
	WO 2000060919	A2	20001019	WO 2000-US9838	20000411
	WO 2000060919	A3	20020103		
	WO 2000060919	B1	20020131		
	W: CA, JP, US				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	JP 2003510012	T2	20030318	JP 2000-610270	20000411
PRAI	US 1999-290000	A	19990412		
	WO 2000-US9838	W	20000411		

AB Multiplex strand displacement amplification of target nucleic acids using  
primer pair sets that are anchored to electronically addressable capture  
sites on a microarray are described. The primer pair sets may be  
individually bound to the capture sites or may comprise a unique branched

primer pair moiety. The anchored primers allow for the simultaneous multiplex capture, amplification and detection of a target nucleic acid derived from any sample source. The system is also less dependent upon the size of the capture oligonucleotides.

RE.CNT 55 THERE ARE 55 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

IT **Phosphorothioate** oligonucleotides

RL: ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)

(base analog contg., **immobilized**, as **primers**;  
multiplex anchored strand displacement amplification on electronically addressable microchip)

IT Oligonucleotides

RL: DEV (Device component use); USES (Uses)

(derivs., spacer in primer **immobilization**; multiplex anchored strand displacement amplification on electronically addressable microchip)

IT Primers (nucleic acid)

RL: ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)

(oligonucleotide analog contg., **immobilized**; multiplex anchored strand displacement amplification on electronically addressable microchip)

IT Amino acids, uses

Peptides, uses

Polyoxyalkylenes, uses

RL: DEV (Device component use); USES (Uses)

(spacer in oligonucleotide **immobilization**; multiplex anchored strand displacement amplification on electronically addressable microchip)

IT 81295-06-9, Restriction endonuclease BsoBI 81295-15-0, Restriction endonuclease BstOI 81295-26-3, Restriction endonuclease HphI 81458-03-9, Restriction endonuclease FokI 81811-55-4, Restriction endonuclease HindII 81811-55-4, Restriction endonuclease HincII 82469-86-1, Restriction endonuclease MraI 84522-63-4, Restriction endonuclease Tth111I 86352-30-9, Restriction endonuclease Fnu4HI 87683-74-7, Restriction endonuclease AccI 92228-43-8, Restriction endonuclease BstXI 95076-97-4, Restriction endonuclease NciI 100900-70-7, Restriction endonuclease BsaI 102227-43-0 115630-38-1, Restriction endonuclease BsrI 121855-06-9, Restriction endonuclease MwoI 122007-72-1, Restriction endonuclease BsmI 122319-85-1, Restriction endonuclease PflMI 126469-98-5, Restriction endonuclease BsmAI 135340-86-2, Restriction endonuclease AlwI 172306-44-4, Restriction endonuclease NspI 219575-51-6, Restriction endonuclease BsrBI  
RL: ARU (Analytical role, unclassified); CAT (Catalyst use); ANST (Analytical study); USES (Uses)

(**cleavage** site for, in strand displacement amplicons;  
multiplex anchored strand displacement amplification on electronically addressable microchip)

IT 1173-82-6, DUTP 22003-12-9, 5-Methyl dCTP 29220-54-0, ATP-.alpha.-S 101515-08-6

RL: MSC (Miscellaneous)

(**immobilized** primers contg.; multiplex anchored strand displacement amplification on electronically addressable microchip)

IT 58-85-5, Biotin 9013-20-1, Streptavidin

RL: DEV (Device component use); USES (Uses)

(oligonucleotide **immobilization** using; multiplex anchored strand displacement amplification on electronically addressable microchip)

IT 25322-68-3, Polyethylene glycol

RL: DEV (Device component use); USES (Uses)

(spacer in oligonucleotide **immobilization**; multiplex anchored strand displacement amplification on electronically addressable microchip)

=> d 111 1-10 bib ab kwic

L11 ANSWER 1 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN  
AN 2003:196911 CAPLUS  
DN 138:232931  
TI Multiplex anchored strand displacement amplification on an electronically addressable microchip  
IN Nerenberg, Michael I.; Edman, Carl F.; Westin, Lorelei P.; Feng, Lana L.; Landis, Geoffrey C.; Sosnowski, Ronald G.  
PA Nanogen/Becton Dickinson Partnership, USA  
SO U.S., 77 pp.  
CODEN: USXXAM  
DT Patent  
LA English  
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 6531302	B1	20030311	US 1999-290000	19990412
	WO 2000060919	A2	20001019	WO 2000-US9838	20000411
	WO 2000060919	A3	20020103		
	WO 2000060919	B1	20020131		

W: CA, JP, US

RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

	JP 2003510012	T2	20030318	JP 2000-610270	20000411
PRAI	US 1999-290000	A	19990412		
	WO 2000-US9838	W	20000411		

AB Multiplex strand displacement amplification of target nucleic acids using primer pair sets that are anchored to electronically addressable capture sites on a microarray are described. The primer pair sets may be individually bound to the capture sites or may comprise a unique branched primer pair moiety. The anchored primers allow for the simultaneous multiplex capture, amplification and detection of a target nucleic acid derived from any sample source. The system is also less dependent upon the size of the capture oligonucleotides.

RE.CNT 55 THERE ARE 55 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

IT **Phosphorothioate** oligonucleotides  
RL: ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)  
(base analog contg., **immobilized**, as **primers**;  
multiplex anchored strand displacement amplification on electronically addressable microchip)

IT Oligonucleotides  
RL: DEV (Device component use); USES (Uses)  
(derivs., spacer in primer **immobilization**; multiplex anchored strand displacement amplification on electronically addressable microchip)

IT Primers (nucleic acid)  
RL: ARG (Analytical reagent use); DEV (Device component use); ANST (Analytical study); USES (Uses)  
(oligonucleotide analog contg., **immobilized**; multiplex anchored strand displacement amplification on electronically addressable microchip)

IT Amino acids, uses  
Peptides, uses  
Polyoxyalkylenes, uses  
RL: DEV (Device component use); USES (Uses)  
(spacer in oligonucleotide **immobilization**; multiplex anchored strand displacement amplification on electronically addressable microchip)

IT 81295-06-9, Restriction endonuclease BsoBI 81295-15-0, Restriction

endonuclease BstOI 81295-26-3, Restriction endonuclease HphI  
 81458-03-9, Restriction endonuclease FokI 81811-55-4, Restriction  
 endonuclease HindII 81811-55-4, Restriction endonuclease HincII  
 82469-86-1, Restriction endonuclease MraI 84522-63-4, Restriction  
 endonuclease Tth111I 86352-30-9, Restriction endonuclease Fnu4HI  
 87683-74-7, Restriction endonuclease AccI 92228-43-8, Restriction  
 endonuclease BstXI 95076-97-4, Restriction endonuclease NciI  
 100900-70-7, Restriction endonuclease BsaI 102227-43-0 115630-38-1,  
 Restriction endonuclease BsrI 121855-06-9, Restriction endonuclease MwoI  
 122007-72-1, Restriction endonuclease BsmI 122319-85-1, Restriction  
 endonuclease PflMI 126469-98-5, Restriction endonuclease BsmAI  
 135340-86-2, Restriction endonuclease AlwI 172306-44-4, Restriction  
 endonuclease NspI 219575-51-6, Restriction endonuclease BsrBI  
 RL: ARU (Analytical role, unclassified); CAT (Catalyst use); ANST  
 (Analytical study); USES (Uses)

(cleavage site for, in strand displacement amplicons;  
 multiplex anchored strand displacement amplification on electronically  
 addressable microchip)

IT 1173-82-6, DUTP 22003-12-9, 5-Methyl dCTP 29220-54-0, ATP-.alpha.-S  
 101515-08-6

RL: MSC (Miscellaneous)

(immobilized primers contg.; multiplex anchored strand  
 displacement amplification on electronically addressable microchip)

IT 58-85-5, Biotin 9013-20-1, Streptavidin

RL: DEV (Device component use); USES (Uses)

(oligonucleotide immobilization using; multiplex anchored  
 strand displacement amplification on electronically addressable  
 microchip)

IT 25322-68-3, Polyethylene glycol

RL: DEV (Device component use); USES (Uses)

(spacer in oligonucleotide immobilization; multiplex anchored  
 strand displacement amplification on electronically addressable  
 microchip)

L11 ANSWER 2 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2002:658292 CAPLUS

DN 137:196646

TI Defined DNA sequences amplifiable with a universal primer pair for use in  
 labeling materials for identification

IN Brown, Tom; Thelwell, Nichola; Maxwell, Paula; Maxwell, Paul; Whiting,  
 Paul

PA Crime Solutions Limited, UK

SO PCT Int. Appl., 23 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002066678	A2	20020829	WO 2002-GB759	20020220
	WO 2002066678	A3	20030530		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,  
 CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,  
 GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,  
 LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,  
 PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,  
 UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU,  
 TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,  
 CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,  
 BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRAI GB 2001-4163 A 20010220

AB A method of uniquely identifying an object by labeling it with a DNA  
 sequence is described. The DNA sequence has a terminal region including a

moiety that can be used to **attach** it to a substrate. Adjacent to this is a sequence by which the DNA can be released from the substrate, such as a restriction enzyme **cleavage** site. The remainder of the DNA is the unique identifier that includes a pair of primer binding sites sepd. by a defined and unique DNA sequence. The DNA may also contain base analogs or have a modified backbone that will prevent degrdn. of the label by nucleases. The DNA may also be single-stranded with the **immobilization** region in the loop of a stem loop structure. The partially double stranded region may serve as a primer for an initial amplification. Amplification and sequencing of the unique sequence identifier can be used to demonstrate ownership.

AB A method of uniquely identifying an object by labeling it with a DNA sequence is described. The DNA sequence has a terminal region including a moiety that can be used to **attach** it to a substrate. Adjacent to this is a sequence by which the DNA can be released from the substrate, such as a restriction enzyme **cleavage** site. The remainder of the DNA is the unique identifier that includes a pair of primer binding sites sepd. by a defined and unique DNA sequence. The DNA may also contain base analogs or have a modified backbone that will prevent degrdn. of the label by nucleases. The DNA may also be single-stranded with the **immobilization** region in the loop of a stem loop structure. The partially double stranded region may serve as a primer for an initial amplification. Amplification and sequencing of the unique sequence identifier can be used to demonstrate ownership.

IT

DNA

Oligonucleotides

**Phosphorothioate** oligonucleotides

RL: ANT (Analyte); TEM (Technical or engineered material use); ANST (Analytical study); USES (Uses)

(as identifying labels; defined DNA sequences amplifiable with universal **primer** pair for use in labeling materials for identification)

IT

80498-17-5, Restriction endonuclease EcoRI

RL: CAT (Catalyst use); USES (Uses)

(**cleavage** site for, in oligonucleotide label; defined DNA sequences amplifiable with universal primer pair for use in labeling materials for identification)

IT

57-88-5D, Cholesterol, nucleic acid conjugates 58-85-5D, Biotin, nucleic acid conjugates 1406-18-4D, Vitamin E, nucleic acid conjugates 15181-41-6D, Thiophosphate, nucleic acid conjugates

RL: TEM (Technical or engineered material use); USES (Uses)

(for **immobilization** of oligonucleotide label; defined DNA sequences amplifiable with universal primer pair for use in labeling materials for identification)

L11 ANSWER 3 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2002:811995 CAPLUS

DN 137:321241

TI Large-scale sequencing by mass spectrometry of extension products of mass-labeled primers

IN Sampson, Jeffrey R.; Myerson, Joel; Tsalenko, Anna M.; Sampas, Nicholas M.; Webb, Peter G.; Yakhini, Zohar H.

PA Agilent Technologies, Inc., USA

SO Eur. Pat. Appl., 48 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 1251136	A2	20021023	EP 2002-252723	20020417
	EP 1251136	A3	20030319		

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR

US 2002182601      A1      20021205      US 2001-836012      20010417  
PRAI US 2001-836012      A      20010417  
US 1998-112437      A2      19980709

AB Methods and reagents are disclosed which provide for more sensitive, more accurate and higher through-put analyses of target nucleic acid sequences. The methods and reagents of the present invention may be generically applied to generally any target nucleic acid sequence and do not require a priori information about the presence, location or identity of mutations in the target nucleic acid sequence. The reagents of the invention are mixts. of oligonucleotide precursors having a high level of coverage and mass no. complexity, and also having tags analyzable by mass spectrometry which are covalently linked to the precursors through **cleavable** bonds. A method is also disclosed for analyzing a target nucleic acid sequence employing the mixts. of oligonucleotide precursors having tags analyzable by mass spectrometry covalently linked to the oligonucleotide precursors through **cleavable** bonds, and chem. or enzymic assays to alter the mass of the oligonucleotide precursors prior to mass spectral anal. The enzymic assay may be a polymerase extension assay or a ligation-based assay. The kits for carrying out the methods of the invention are also disclosed.

AB Methods and reagents are disclosed which provide for more sensitive, more accurate and higher through-put analyses of target nucleic acid sequences. The methods and reagents of the present invention may be generically applied to generally any target nucleic acid sequence and do not require a priori information about the presence, location or identity of mutations in the target nucleic acid sequence. The reagents of the invention are mixts. of oligonucleotide precursors having a high level of coverage and mass no. complexity, and also having tags analyzable by mass spectrometry which are covalently linked to the precursors through **cleavable** bonds. A method is also disclosed for analyzing a target nucleic acid sequence employing the mixts. of oligonucleotide precursors having tags analyzable by mass spectrometry covalently linked to the oligonucleotide precursors through **cleavable** bonds, and chem. or enzymic assays to alter the mass of the oligonucleotide precursors prior to mass spectral anal. The enzymic assay may be a polymerase extension assay or a ligation-based assay. The kits for carrying out the methods of the invention are also disclosed.

IT Phosphates, analysis  
RL: ARU (Analytical role, unclassified); RCT (Reactant); ANST (Analytical study); RACT (Reactant or reagent)  
(**phosphorothioates**, nucleoside triphosphates contg., in prepn. modified **primer** extension products for mass spectrometry; large-scale sequencing by mass spectrometry of extension products of mass-labeled primers)

IT Linking agents  
(photolabile, for **attachment** of mass label to primer; large-scale sequencing by mass spectrometry of extension products of mass-labeled primers)

IT 9012-90-2, DNA polymerase  
RL: ARU (Analytical role, unclassified); CAT (Catalyst use); ANST (Analytical study); USES (Uses)  
(for **cleavage** of primer extension products in DNA sequencing; large-scale sequencing by mass spectrometry of extension products of mass-labeled primers)

L11 ANSWER 4 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2002:656066 CAPLUS

DN 137:164662

TI Mutation analysis by mass spectrometry using photocleavable primers in mutation-dependent primer extension reaction

IN Kostrzewa, Markus; Froehlich, Thomas; Wenzel, Thomas; Jaeschke, Andreas; Hausch, Felix

PA Bruker-Saxonia Analytik G.m.b.H., Germany

SO Eur. Pat. Appl., 11 pp.



CODEN: EPXXDW

DT Patent  
LA English  
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 1234888	A2	20020828	EP 2002-2521	20020201
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
	DE 10108453	A1	20020912	DE 2001-10108453	20010222
	US 2002187493	A1	20021212	US 2002-79043	20020220
PRAI	DE 2001-10108453	A	20010222		

AB The invention relates to a method of a mass-spectrometric anal. of known mutation sites in the genome, such as single nucleotide polymorphisms (SNPs), using the method of restricted mutation-dependent primer extension. Mutation-dependent primer extension can be carried out simply by using four ddNTPs, such as .alpha.-S-ddNTP. The invention consists of the use of primers with a photocleavable linker, a photocleavable .beta.-cyanoethylphosphoramidites (o-nitrobenzyl derivs.) contg. .alpha. 1,3-propanediol moiety instead of a sugar unit. The photocleavable linker can be used a building block, replacing a regular nucleoside at any position of the primer during primer synthesis. The linker creates a gap in a DNA strand which is almost the same size as a natural DNA building block (nucleoside). The linker forms a bridge over a base pair without inhibiting hybridization or enzymic extension. However, the linker allows the primers to be **cleaved** after extension in order to obtain short DNA fragments which can be more easily detected on the mass spectrometer. The photocleavable linker-tagged primer can also be bonded to a pos. charged group from quaternary ammonium salts to the .alpha.-S-ddNTP terminal positions or to the phosphorothioate nucleoside building blocks. Neutralization of the sugar/thiophosphate backbone by alkylation can increase the detection sensitivity significantly in the mass spectrometric anal. The charge-neutralized variations of the equal-no.-nucleotide primer extension method introduced from these novel compds. within the extension primers enables flexible design of multiplex genotyping reactions. The photocleavable primers can be bonded to a 5' amino group, or biotinylated and bonded to other streptavidin-coated surfaces to facilitate product isolation and purifn.

AB The invention relates to a method of a mass-spectrometric anal. of known mutation sites in the genome, such as single nucleotide polymorphisms (SNPs), using the method of restricted mutation-dependent primer extension. Mutation-dependent primer extension can be carried out simply by using four ddNTPs, such as .alpha.-S-ddNTP. The invention consists of the use of primers with a photocleavable linker, a photocleavable .beta.-cyanoethylphosphoramidites (o-nitrobenzyl derivs.) contg. .alpha. 1,3-propanediol moiety instead of a sugar unit. The photocleavable linker can be used a building block, replacing a regular nucleoside at any position of the primer during primer synthesis. The linker creates a gap in a DNA strand which is almost the same size as a natural DNA building block (nucleoside). The linker forms a bridge over a base pair without inhibiting hybridization or enzymic extension. However, the linker allows the primers to be **cleaved** after extension in order to obtain short DNA fragments which can be more easily detected on the mass spectrometer. The photocleavable linker-tagged primer can also be bonded to a pos. charged group from quaternary ammonium salts to the .alpha.-S-ddNTP terminal positions or to the phosphorothioate nucleoside building blocks. Neutralization of the sugar/thiophosphate backbone by alkylation can increase the detection sensitivity significantly in the mass spectrometric anal. The charge-neutralized variations of the equal-no.-nucleotide primer extension method introduced from these novel compds. within the extension primers enables flexible design of multiplex genotyping reactions. The photocleavable primers can be bonded to a 5' amino group, or biotinylated and bonded to other streptavidin-coated surfaces to facilitate product isolation and purifn.

IT Nucleotides, uses  
 RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)  
 (2',3'-dideoxyribo-, triphosphates, .alpha.-S, as terminator; mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

IT Functional groups  
 (benzyl group, o-nitro, photocleavage site; mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

IT UV radiation  
 (for photocleavable primer **cleavage**; mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

IT Genotyping (method)  
**Immobilization**, molecular  
 Mutation  
 Time-of-flight mass spectrometry  
 (mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

IT Primers (nucleic acid)  
 RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
 (mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

IT Alkylation  
 (of sugar/thiophosphate backbone for neutralization, for MALDI mass spectrometry; mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

IT Catalysts  
 (photochem., photocleavable primer **cleaved** by UV light irradiation; mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

IT Quaternary ammonium compounds, biological studies  
 RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
 (photocleavable primer pos. charge group derived from; mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

IT **Phosphorothioate** oligodeoxyribonucleotides  
 RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
 (photocleavable; mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

IT Laser ionization mass spectrometry  
 (photo-desorption, matrix-assisted; mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

IT Laser desorption mass spectrometry  
 (photoionization, matrix-assisted; mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

IT Amide group  
 (primer bonded to; mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

IT Nucleic acid amplification (method)  
 (primer extension, using photocleavable primers; mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

IT Sulfhydryl group  
(reactive functional group for covalent linking; mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

IT Genetic polymorphism  
(single nucleotide, detection of; mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

IT PCR (polymerase chain reaction)  
(using photocleavable primers; mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

IT 3695-85-0  
RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(as mass spectrometry matrix; mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

IT 363626-65-7, 1,3-Propanediol, 1-(2-nitrophenyl)-, bis(dihydrogen phosphate) (ester)  
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
(as photocleavable linker in primer; mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

IT 58-85-5, Biotin  
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
(primer labeled with; mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

IT 504-63-2, 1,3-Propanediol  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(replacing sugar unit in photocleavable nucleoside; mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

IT 9013-20-1, Streptavidin  
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
(surface coated with, for biotinylated primer **immobilization**; mutation anal. by mass spectrometry using photolytically **cleavable** primers in mutation-dependent primer extension reaction)

L11 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2001:798467 CAPLUS

DN 135:353694

TI Method for highly parallel analysis of genetic polymorphisms and DNA methylation

IN Berlin, Kurt; Gut, Ivo Glynne

PA Epigenomics A.-G., Germany

SO PCT Int. Appl., 33 pp.

CODEN: PIXXD2

DT Patent

LA German

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2001081620	A2	20011101	WO 2001-DE1607	20010425
	WO 2001081620	A3	20030213		
	W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT,			

LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU,  
SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN,  
YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM  
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,  
DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF,  
BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

DE 10021204 A1 20011108 DE 2000-10021204 20000425

EP 1309728 A2 20030514 EP 2001-942981 20010425

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,  
IE, SI, LT, LV, FI, RO, MK, CY, AL, TR

PRAI DE 2000-10021204 A 20000425

WO 2001-DE1607 W 20010425

AB The invention relates to a method for the highly parallel characterization of polymorphisms, esp. SNPs, which can be used for the simultaneous or sep. detection of DNA methylations. A set of probes that is provided with at least one detectable marking characteristic of the corresponding probe is bound to an addressed surface, the bond of the probes to the surface being photochem., chem. or enzymically **cleavable**. A nucleic acid to be analyzed is then bound to said probes, the probes are modified in an allele-specific enzymic reaction and a part of the probes that is irrelevant for the anal. of the allele-specific reaction is removed. The allele-specific products are analyzed by means of the detectable markings and the alleles present in the analyzed nucleic acid probe are detd. Thus, the methylation status of the first methylation position in exon 14 of the factor VIII gene was detd. using MALDI-TOF mass spectrometry. The genomic DNA was first treated with bisulfite then a fragment of exon 14 was amplified with PCR. In the first step of the polymorphism assay, the **primer** TCTATTTACTTCATTCCACTTAASt\*sC (s indicates a **phosphorothioate** linkage; \* indicates the presence of a quaternary ammonium group on the T) was extended with DNA polymerase in the presence of the amplicon and .alpha.-S-ddATP and .alpha.-S-ddGTP. Depending on the methylation status of the exon, either TCTATTTACTTCATTCCACTTAASt\*sCsA or TCTATTTACTTCATTCCACTTAASt\*sCsG will be produced. After primer extension, the reaction mixt. was treated with 5'-phosphodiesterase. This produces AsT\*sCsA and AsT\*sCsG which are reacted with Me iodide then subjected to MALDI-TOF mass spectrometry.

AB The invention relates to a method for the highly parallel characterization of polymorphisms, esp. SNPs, which can be used for the simultaneous or sep. detection of DNA methylations. A set of probes that is provided with at least one detectable marking characteristic of the corresponding probe is bound to an addressed surface, the bond of the probes to the surface being photochem., chem. or enzymically **cleavable**. A nucleic acid to be analyzed is then bound to said probes, the probes are modified in an allele-specific enzymic reaction and a part of the probes that is irrelevant for the anal. of the allele-specific reaction is removed. The allele-specific products are analyzed by means of the detectable markings and the alleles present in the analyzed nucleic acid probe are detd. Thus, the methylation status of the first methylation position in exon 14 of the factor VIII gene was detd. using MALDI-TOF mass spectrometry. The genomic DNA was first treated with bisulfite then a fragment of exon 14 was amplified with PCR. In the first step of the polymorphism assay, the **primer** TCTATTTACTTCATTCCACTTAASt\*sC (s indicates a **phosphorothioate** linkage; \* indicates the presence of a quaternary ammonium group on the T) was extended with DNA polymerase in the presence of the amplicon and .alpha.-S-ddATP and .alpha.-S-ddGTP. Depending on the methylation status of the exon, either TCTATTTACTTCATTCCACTTAASt\*sCsA or TCTATTTACTTCATTCCACTTAASt\*sCsG will be produced. After primer extension, the reaction mixt. was treated with 5'-phosphodiesterase. This produces AsT\*sCsA and AsT\*sCsG which are reacted with Me iodide then subjected to MALDI-TOF mass spectrometry.

ST SNP DNA methylation **immobilized** labeled releasable probe mass spectrometry

IT Probes (nucleic acid)

RL: ARG (Analytical reagent use); BPR (Biological process); BSU

(Biological study, unclassified); ANST (Analytical study); BIOL  
(Biological study); PROC (Process); USES (Uses)  
(labeled, **immobilized**, releasable; method for highly parallel  
anal. of genetic polymorphisms and DNA methylation)

IT 4044-65-9, Phenylene-1,4-diisothiocyanate 85419-94-9 134978-97-5  
162827-98-7

RL: RCT (Reactant); RACT (Reactant or reagent)  
(oligonucleotide **immobilization** and; method for highly  
parallel anal. of genetic polymorphisms and DNA methylation)

L11 ANSWER 6 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1999:655898 CAPLUS

DN 131:282375

TI Improvement of mass resolution and accuracy in screening nucleic acids for  
polymorphisms by mass spectrometric analysis

IN Monforte, Joseph Albert; Shaler, Thomas Andrew; Tan, Yuping; Becker,  
Christopher Hank

PA Genetrace Systems Inc., USA

SO U.S., 32 pp., Cont.-in-part of U.S. Ser. No. 715,582.

CODEN: USXXAM

DT Patent

LA English

FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 5965363	A	19991012	US 1996-759993	19961202
	CA 2301875	AA	19980326	CA 1997-2301875	19970919
	WO 9812355	A1	19980326	WO 1997-US17101	19970919
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, US, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	AU 9745916	A1	19980414	AU 1997-45916	19970919
	US 6566055	B1	20030520	US 1998-89730	19980603
PRAI	US 1996-715582	A2	19960919		
	US 1996-32369P	P	19961202		
	US 1996-759993	A	19961202		
	WO 1997-US17101	W	19970919		

AB This invention relates to methods for screening nucleic acids for  
polymorphisms by analyzing amplified target nucleic acids using mass  
spectrometric techniques and to procedures for improving mass resolu. and  
mass accuracy of these methods of detecting polymorphisms. The methods  
for improving resolu. and accuracy include (1) reducing the length of the  
target nucleic acid by removing regions flanking the region of interest  
and (2) purifn. of single-stranded DNA. Methods for reducing the length  
include use of PCR primer contg. restriction endonuclease sites. Methods  
for prep. single-stranded DNA of required purity include use of PCR  
primers contg. exonuclease-resistant regions followed by digestion with  
exonuclease. These same primers may be labeled with a binding pair, such  
as biotin or streptavidin, which allows **immobilization** of the  
amplified product. The method was applied to anal. of a tetranucleotide  
repeat polymorphism in human gene TH01. A biotinylated,  
2'-deoxythymidine-5'-(S)-**phosphorothioate**-contg. **primer**  
was employed. After amplification, the double-stranded DNA was denatured  
and the target was released by incubation with AgNO3.

RE.CNT 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

AB This invention relates to methods for screening nucleic acids for  
polymorphisms by analyzing amplified target nucleic acids using mass  
spectrometric techniques and to procedures for improving mass resolu. and

mass accuracy of these methods of detecting polymorphisms. The methods for improving resolu. and accuracy include (1) reducing the length of the target nucleic acid by removing regions flanking the region of interest and (2) purifn. of single-stranded DNA. Methods for reducing the length include use of PCR primer contg. restriction endonuclease sites. Methods for prepg. single-stranded DNA of required purity include use of PCR primers contg. exonuclease-resistant regions followed by digestion with exonuclease. These same primers may be labeled with a binding pair, such as biotin or streptavidin, which allows immobilization of the amplified product. The method was applied to anal. of a tetranucleotide repeat polymorphism in human gene THO1. A biotinylated, 2'-deoxythymidine-5'-(S)-phosphorothioate-contg. primer was employed. After amplification, the double-stranded DNA was denatured and the target was released by incubation with AgNO3.

IT Primers (nucleic acid)

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(cleavable, exonuclease-resistant; improvement of mass resolu. and accuracy in screening nucleic acids for polymorphisms by mass spectrometric anal.)

L11 ANSWER 7 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1998:197643 CAPLUS

DN 128:254923

TI Improvement of mass resolution and accuracy in screening nucleic acids for polymorphisms by mass spectrometric analysis

IN Monforte, Joseph Albert; Shaler, Thomas A.; Tan, Yuping; Becker, Christopher H.

PA Genetrace Systems, USA; Monforte, Joseph Albert; Shaler, Thomas A.; Tan, Yuping; Becker, Christopher H.

SO PCT Int. Appl., 84 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9812355	A1	19980326	WO 1997-US17101	19970919
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, US, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	US 5965363	A	19991012	US 1996-759993	19961202
	CA 2301875	AA	19980326	CA 1997-2301875	19970919
	AU 9745916	A1	19980414	AU 1997-45916	19970919
PRAI	US 1996-715582	A1	19960919		
	US 1996-32369P	P	19961202		
	US 1996-759993	A1	19961202		
	WO 1997-US17101	W	19970919		
AB	This invention relates to methods for screening nucleic acids for polymorphisms by analyzing amplified target nucleic acids using mass spectrometric techniques and to procedures for improving mass resolu. and mass accuracy of these methods of detecting polymorphisms. The methods for improving resolu. and accuracy include (1) reducing the length of the target nucleic acid by removing regions flanking the region of interest and (2) purifn. of single-stranded DNA. Methods for reducing the length include use of PCR primer contg. restriction endonuclease sites. Methods for prepg. single-stranded DNA of required purity include use of PCR primers contg. exonuclease-resistant regions followed by digestion with exonuclease. These same primers may be labeled with a binding pair, such				

as biotin or streptavidin, which allows **immobilization** of the amplified product. The method was applied to anal. of a tetranucleotide repeat polymorphism in human gene TH01. A biotinylated, 2'-deoxythymidine-5'-(S)-**phosphorothioate-contg. primer** was employed. After amplification, the double-stranded DNA was denatured and the target was released by incubation with AgNO<sub>3</sub>.

RE.CNT 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

AB This invention relates to methods for screening nucleic acids for polymorphisms by analyzing amplified target nucleic acids using mass spectrometric techniques and to procedures for improving mass resoln. and mass accuracy of these methods of detecting polymorphisms. The methods for improving resoln. and accuracy include (1) reducing the length of the target nucleic acid by removing regions flanking the region of interest and (2) purifn. of single-stranded DNA. Methods for reducing the length include use of PCR primer contg. restriction endonuclease sites. Methods for prepg. single-stranded DNA of required purity include use of PCR primers contg. exonuclease-resistant regions followed by digestion with exonuclease. These same primers may be labeled with a binding pair, such as biotin or streptavidin, which allows **immobilization** of the amplified product. The method was applied to anal. of a tetranucleotide repeat polymorphism in human gene TH01. A biotinylated, 2'-deoxythymidine-5'-(S)-**phosphorothioate-contg. primer** was employed. After amplification, the double-stranded DNA was denatured and the target was released by incubation with AgNO<sub>3</sub>.

IT Primers (nucleic acid)

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(**cleavable**, exonuclease-resistant; improvement of mass resoln. and accuracy in screening nucleic acids for polymorphisms by mass spectrometric anal.)

L11 ANSWER 8 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1997:454756 CAPLUS

DN 127:202056

TI Cofactor-assisted self-**cleavage** in DNA libraries with a 3'-5'-phosphoramidate bond

AU Burmeister, Jens; von Kiedrowski, Gunter; Ellington, Andrew D.

CS Lehrstuhl Organische Chemie I Universitat, Bochum, D-44801, Germany

SO Angewandte Chemie, International Edition in English (1997), 36(12), 1321-1324

CODEN: ACIEAY; ISSN: 0570-0833

PB Wiley-VCH

DT Journal

LA English

AB 3'-5'-Phosphoramidate bond-contg. DNA sequences capable of catalyzing cofactor-assisted self-**cleavage** were obtained by in vitro selection from an oligonucleotide library contg. a randomized 72-mer sequence. The method involved prepn. of **immobilized** phosphoramidate-contg. randomized 72-mer. The 72-mer was then allowed to react in presence of dansylated trimer, hexameric template, and magnesium. Reaction produced a pool of catalytic 72-mers which was amplified for the next round of selection. Neg. selection (removal of those DNA sequences that were released via uncatalyzed hydrolysis) was also utilized. Cloning and sequencing of PCR products from the last rounds revealed a single dominating clone. The secondary structure was predicted and showed tight folding around the const. primer regions. Closer examn. of the cloned sequence showed that it catalyzes the hydrolysis (not transphosphorylation) of an internal 3'-5'-phosphoramidate bond in the presence of a specific trimeric cofactor.

TI Cofactor-assisted self-**cleavage** in DNA libraries with a 3'-5'-phosphoramidate bond

AB 3'-5'-Phosphoramidate bond-contg. DNA sequences capable of catalyzing cofactor-assisted self-**cleavage** were obtained by in vitro

selection from an oligonucleotide library contg. a randomized 72-mer sequence. The method involved prepn. of **immobilized** phosphoramidate-contg. randomized 72-mer. The 72-mer was then allowed to react in presence of dansylated trimer, hexameric template, and magnesium. Reaction produced a pool of catalytic 72-mers which was amplified for the next round of selection. Neg. selection (removal of those DNA sequences that were released via uncatalyzed hydrolysis) was also utilized. Cloning and sequencing of PCR products from the last rounds revealed a single dominating clone. The secondary structure was predicted and showed tight folding around the const. primer regions. Closer examn. of the cloned sequence showed that it catalyzes the hydrolysis (not transphosphorylation) of an internal 3'-5'-phosphoramidate bond in the presence of a specific trimeric cofactor.

- ST self **cleaving** phosphoramidate bond DNA library; deoxyribozyme  
self **cleaving** phosphoramidate bond
- IT Ribozymes  
RL: NUU (Other use, unclassified); SPN (Synthetic preparation); PREP (Preparation); USES (Uses)  
(deoxy; in vitro selection and characterization of cofactor-assisted self-**cleaving** phosphoramidate-contg. deoxyribozymes from DNA libraries)
- IT Hydrolysis catalysts  
Hydrolysis kinetics  
Nucleic acid library  
(in vitro selection and characterization of cofactor-assisted self-**cleaving** phosphoramidate-contg. deoxyribozymes from DNA libraries)
- IT Secondary structure  
(predicted; in vitro selection and characterization of cofactor-assisted self-**cleaving** phosphoramidate-contg. deoxyribozymes from DNA libraries)
- IT 194555-77-6 194555-78-7  
RL: NUU (Other use, unclassified); RCT (Reactant); RACT (Reactant or reagent); USES (Uses)  
(PCR **primer**; in vitro selection and characterization of cofactor-assisted self-**cleaving phosphoramidate** -contg. deoxyribozymes from DNA libraries)
- IT 94854-99-6  
RL: NUU (Other use, unclassified); USES (Uses)  
(hexameric template; in vitro selection and characterization of cofactor-assisted self-**cleaving** phosphoramidate-contg. deoxyribozymes from DNA libraries)
- IT 7439-95-4, Magnesium, uses  
RL: NUU (Other use, unclassified); USES (Uses)  
(in vitro selection and characterization of cofactor-assisted self-**cleaving** phosphoramidate-contg. deoxyribozymes from DNA libraries)
- IT 194675-13-3P  
RL: NUU (Other use, unclassified); SPN (Synthetic preparation); PREP (Preparation); USES (Uses)  
(in vitro selection and characterization of cofactor-assisted self-**cleaving** phosphoramidate-contg. deoxyribozymes from DNA libraries)
- IT 194675-86-0  
RL: NUU (Other use, unclassified); RCT (Reactant); RACT (Reactant or reagent); USES (Uses)  
(**phosphoramidate**-contg. PCR **primer**; in vitro selection and characterization of cofactor-assisted self-**cleaving phosphoramidate**-contg. deoxyribozymes from DNA libraries)
- IT 194554-76-2, DNA (synthetic 72-nucleotide)  
RL: NUU (Other use, unclassified); RCT (Reactant); RACT (Reactant or reagent); USES (Uses)  
(randomized 72-mer library; in vitro selection and characterization of



cofactor-assisted self-cleaving phosphoramidate-contg.  
deoxyribozymes from DNA libraries)

IT 194602-90-9

RL: NUU (Other use, unclassified); RCT (Reactant); RACT (Reactant or reagent); USES (Uses)

(trimeric cofactor; in vitro selection and characterization of cofactor-assisted self-cleaving phosphoramidate-contg. deoxyribozymes from DNA libraries)

L11 ANSWER 9 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1997:88765 CAPLUS

DN 126:100252

TI **Cleavable** primers and oligonucleotide sizing by mass spectrometry and application to DNA sequencing and single base fingerprinting

IN Monforte, Joseph Albert; Becker, Christopher Hank; Shaler, Thomas Andrew; Pollart, Daniel Joseph

PA Sri International, USA

SO PCT Int. Appl., 111 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9637630	A1	19961128	WO 1996-US6116	19960430
	W: AU, CA, CN, JP, KR				
	RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	US 5700642	A	19971223	US 1995-445751	19950522
	US 5830655	A	19981103	US 1996-639363	19960426
	AU 9656352	A1	19961211	AU 1996-56352	19960430
	AU 695705	B2	19980820		
	EP 828855	A1	19980318	EP 1996-913305	19960430
	EP 828855	B1	19991215		
	R: DE, FR, GB, IT, NL				
	JP 11505127	T2	19990518	JP 1996-535680	19960430
	JP 3437184	B2	20030818		
PRAI	US 1995-445751	A	19950522		
	US 1996-639363	A	19960426		
	WO 1996-US6116	W	19960430		

AB The present invention provides modified oligonucleotide primers designed to incorporate a **cleavable** moiety so that a (3') portion of the primer (linked to an extension product) can be released from an upstream (5') portion of the primer. The **cleavable** site may consist of altered internucleoside linkages, e.g. dialkoxysilane, 3'-(or 5'-) S-phosphorothioate, 3'-(or 5'-) N-phosphoramidate, or may be uracil or ribose. Upon selective **cleavage** of the **cleavable** site, primer extension products that contain about five or fewer base pairs of the primer sequence are released, to provide more useful sizing and sequence information per fragment than extension products contg. the entire primer. **Cleavage** may be enzymic, with 5'.fwdarw.3'-exonuclease, or chem. The oligonucleotides are sized by mass spectrometry, esp. matrix-assisted laser desorption ionization-time-of-flight mass spectrometry. The oligonucleotide primer may be modified (with a biotin tag, for example) so that it can be **immobilized**. The synthesis of a variety of **cleavable** dinucleotides and their incorporation into oligodeoxynucleotides, **immobilization** of the modified oligodeoxynucleotides, and selective **cleavage** and mass spectral anal. of the products was discussed. The method was applied to detection of point mutations in a model target sequence.

TI **Cleavable** primers and oligonucleotide sizing by mass spectrometry and application to DNA sequencing and single base fingerprinting

AB The present invention provides modified oligonucleotide primers designed

to incorporate a **cleavable** moiety so that a (3') portion of the primer (linked to an extension product) can be released from an upstream (5') portion of the primer. The **cleavable** site may consist of altered internucleoside linkages, e.g. dialkoxysilane, 3'- (or 5'-) S-phosphorothioate, 3'- (or 5'-) N-phosphoramidate, or may be uracil or ribose. Upon selective **cleavage** of the **cleavable** site, primer extension products that contain about five or fewer base pairs of the primer sequence are released, to provide more useful sizing and sequence information per fragment than extension products containing the entire primer. **Cleavage** may be enzymic, with 5'-phosphatase, 3'-exonuclease, or chem. The oligonucleotides are sized by mass spectrometry, esp. matrix-assisted laser desorption ionization-time-of-flight mass spectrometry. The oligonucleotide primer may be modified (with a biotin tag, for example) so that it can be **immobilized**. The synthesis of a variety of **cleavable** dinucleotides and their incorporation into oligodeoxynucleotides, **immobilization** of the modified oligodeoxynucleotides, and selective **cleavage** and mass spectral anal. of the products was discussed. The method was applied to detection of point mutations in a model target sequence.

- ST oligonucleotide **cleavable** sizing mass spectrometry; DNA sequence  
detn mass spectrometry; single base DNA fingerprinting mass spectrometry
- IT Genetic methods  
(adenine fingerprinting of DNA; **cleavable** primers and  
oligonucleotide sizing by mass spectrometry and application to DNA  
sequencing and single base fingerprinting)
- IT Nucleotides, biological studies  
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES  
(Uses)  
(**cleavable** linkage; **cleavable** primers and  
oligonucleotide sizing by mass spectrometry and application to DNA  
sequencing and single base fingerprinting)
- IT DNA sequence analysis  
Mass spectrometry  
Time-of-flight mass spectrometry  
(**cleavable** primers and oligonucleotide sizing by mass  
spectrometry and application to DNA sequencing and single base  
fingerprinting)
- IT Primers (nucleic acid)  
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); SPN  
(Synthetic preparation); ANST (Analytical study); BIOL (Biological study);  
PREP (Preparation); USES (Uses)  
(**cleavable** primers and oligonucleotide sizing by mass  
spectrometry and application to DNA sequencing and single base  
fingerprinting)
- IT **Immobilization**, biochemical  
(of primers; **cleavable** primers and oligonucleotide sizing by  
mass spectrometry and application to DNA sequencing and single base  
fingerprinting)
- IT Laser ionization mass spectrometry  
(photodesorption, matrix-assisted; **cleavable** primers and  
oligonucleotide sizing by mass spectrometry and application to DNA  
sequencing and single base fingerprinting)
- IT Laser desorption mass spectrometry  
(photoionization, matrix-assisted; **cleavable** primers and  
oligonucleotide sizing by mass spectrometry and application to DNA  
sequencing and single base fingerprinting)
- IT Genetic methods  
(single base DNA fingerprinting; **cleavable** primers and  
oligonucleotide sizing by mass spectrometry and application to DNA  
sequencing and single base fingerprinting)
- IT Oligodeoxyribonucleotides  
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST  
(Analytical study); BIOL (Biological study); USES (Uses)  
(single dialkoxysilane linkage-contg.; **cleavable** primers and

oligonucleotide sizing by mass spectrometry and application to DNA sequencing and single base fingerprinting)

IT Oligodeoxyribonucleotides  
 RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
 (single **phosphoramidate** linkage-contg.; **cleavable primers** and oligonucleotide sizing by mass spectrometry and application to DNA sequencing and single base fingerprinting)

IT Oligodeoxyribonucleotides  
 RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
 (single thiophosphate linkage-contg.; **cleavable primers** and oligonucleotide sizing by mass spectrometry and application to DNA sequencing and single base fingerprinting)

IT 50-69-1, Ribose 66-22-8, Uracil, biological studies  
 RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)  
 (**cleavable** linkage; **cleavable primers** and oligonucleotide sizing by mass spectrometry and application to DNA sequencing and single base fingerprinting)

IT 185831-49-6D, biotin conjugate  
 RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
 (**cleavable primer**; **cleavable primers** and oligonucleotide sizing by mass spectrometry and application to DNA sequencing and single base fingerprinting)

IT 185831-48-5  
 RL: PEP (Physical, engineering or chemical process); PROC (Process)  
 (**cleavable primer**; **cleavable primers** and oligonucleotide sizing by mass spectrometry and application to DNA sequencing and single base fingerprinting)

IT 79121-99-6, 5'.fwdarw.3'-Exonuclease  
 RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
 (**cleavable primers** and oligonucleotide sizing by mass spectrometry and application to DNA sequencing and single base fingerprinting)

IT 1173-82-6, DUTP 59088-21-0, Uracil DNA-glycosylase  
 RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
 (in adenine fingerprinting; **cleavable primers** and oligonucleotide sizing by mass spectrometry and application to DNA sequencing and single base fingerprinting)

IT 9012-90-2, DNA polymerase 9015-85-4, DNA ligase  
 RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
 (primer extension with; **cleavable primers** and oligonucleotide sizing by mass spectrometry and application to DNA sequencing and single base fingerprinting)

L11 ANSWER 10 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1994:2245 CAPLUS

DN 120:2245

TI Automated sequencing of large fragments of DNA using bases labelled with **cleavable** reporter groups

IN Rosenthal, Andre; Brenner, Sydney

PA Medical Research Council, UK

SO PCT Int. Appl., 74 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI	WO 9321340	A1	19931028	WO 1993-GB848	19930422
	W: AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, VN				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	AU 9340208	A1	19931118	AU 1993-40208	19930422
	EP 640146	A1	19950301	EP 1993-909381	19930422
	EP 640146	B1	19971029		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
	JP 07507681	T2	19950831	JP 1993-518158	19930422
	AT 159766	E	19971115	AT 1993-909381	19930422
	ES 2110604	T3	19980216	ES 1993-909381	19930422
	US 6087095	A	20000711	US 1994-325224	19941209
PRAI	GB 1992-8733	A	19920422		
	WO 1993-GB848	A	19930422		

AB A primer extension method for sequencing DNA that involves directly testing the incorporation of different bases is described. The testing is carried out using a **cleavable** reporter group and the sugar phosphate may also be blocked with a **cleavable** group to prevent secondary incorporation; other forms of inhibition of chain elongation may also be used. The sample is incubated in turn with single labeled nucleotides and any incorporation is measured and the incorporated base is then removed or deprotected and the next base is detd. Removal of the labeled and blocked base may be carried out with exonuclease III in which case the 3'-terminal base of the **primer** is linked with an exonuclease resistant bond such as a **phosphorothioate**. Several variants of the method are described and demonstrated.

TI Automated sequencing of large fragments of DNA using bases labelled with **cleavable** reporter groups

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IT Deoxyribonucleic acids

RL: BIOL (Biological study)

(**immobilized**, sequence detn. by sequential testing of incorporation of labeled bases in primer extension of)

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